Sub C2x.d cont'd
B3 Cont

second exogenes obligatorily segregate to different gametes.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-9 and 11-57 are pending in this case. Claims 1-9, 11-46, 48 and 52-54 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 47, 49-51 and 55-57 are examined. Claims 47, 49-51 and 55-57 have been rejected. Claims 47 and 49-51 and 55 have now been amended.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 47 and 49, 56 and 57 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

In particular, the Examiner states that Applicants arguments that the recombination events described by Qin et al. and Golic et al. would not pose a problem in the present invention are not convincing.

Applicant submits that the recombination events described by Qin et al. and Golic would not exist in any steps used by the method of the present invention nor would they exist in the plants generated according to the teachings of the present invention.

Claims 47 and 49 and claims 56 and 57 which directly depend therefrom (respectively) are directed at methods of generating exogenic allelism (EA) in a plant. Such exogenic allelism is generated by providing a first and a second isogenic plants homozygous for an expression cassette which includes site specific recombinase sequences flanking transcribable sequences. As described in the specification, the first and second isogenic plants homozygous for an expression cassette are in fact identical progeny of a single transformation event. As such, the first and second plants include the expression cassette integrated

within the same site of the same chromosome and as such are isogenic.

In the embodiment illustrated in Figure 1, and claimed in claims 47 and 56, a first isogenic plant is subjected to a recombination event using a recombinase gene which is introduced via transformation techniques or preferably via out-crossing with a recombinase expressing organism which does not contain the expression cassette, but which is preferably isogenic to the first plant. The other isogenic plant is not subjected to recombination but rather crossed with the first isogenic plant resultant from the recombination event. As is illustrated in Figure 1, the recombinase gene introduced into the first plant is selected out either following generation of the progeny of the recombination event, or following the last crossing event, which generates the plant exhibiting exogenic allelism. In any case, selection of a recombinase minus plant can be effected using prior art techniques which utilize, for example, recombinase specific probe sequences.

In the interest of expediting prosecution in this case, claim 47 has now been amended to recite a step of "selfing a plant resultant from step (b) and selecting progeny which is recombinase minus". Such a selection step, which is illustrated Figure 1 of the instant application, eliminates the recombinase gene and as such traverses the problems described by Qin et al. and Golic et al.

It should be noted however, that such a step is not critical at the various generation stages and in fact can be effected concomitantly with the selection of plants exhibiting EA.

In addition, it should also be noted that since the plant subjected to recombination according to the method illustrated in Figure 1 can be a heterozygous plant including a single copy of the cassette (step II of Figure 1), inter-chromosomal recombination events are not possible at any stage of the generation process.

In the embodiment illustrated in Figure 2 and claimed in claims 49 and 57, such selection can be effected following generation of each excised plant or at the end of the generation process.

To further clarify this point, Applicant has chosen to amend claim 49 to include limitations of selfing both plants (first and second) and selecting only the progeny which is recombinase minus.

It should be noted that the plants generated according to the teachings of the present invention are characterized by exogenic allelism which enables 100 % segregation of two exogenes into separate gametes. Although the methods of generating such plants may not be 100 % successful at all time, as is the case with virtually all molecular and genetic techniques, it is well accepted that successful implementation of genetic methodology is oftentimes measured by single digit percentages.

For example, plant transformation techniques which generate transformants in 1-20 % of the progeny (depending on the transformation method and the species transformed) are considered highly successful and of utility and as such are used routinely and described regularly as methods of choice in numerous patent documents.

Thus, even in light of the unpredictability typical of this art, the large numbered progeny produced by even a single plant stipulates the methodology of the present invention can be considered successful even in case where only 10 % of the progeny obtained will grow into plants characterized by exogenic allelism. However, since reversal of the phenotype generated by EA results from simple cross of two homozygous parents, 100% of the offspring will exhibit reversal of the EA associated phenotype.

It should be emphasized that although the problems described by Qin et al. and Golic et al. can potentially limit the number of usable progeny plants (plants exhibiting EA), such plants, when generated for the purpose of reversible male sterility, would not be subjected to further recombinase excisions in order to recover fertility and as such, would not exhibit the recombinase problems described above.

Thus, in sharp contrast to prior art methods which employ recombinases <u>for reversing an established phenotype</u>, the EA phenotype of the plants

generated according to the teachings of the present invention can be reversed simply by using plant breeding techniques thus negating the possibility of uncontrollable excision/recombination events.

Thus, since the excision and breeding methodology employed by the present invention is less affected by the excision problems described by Qin et al. and Golic et al., a high degree of success can be expected when employing the methodology of the present invention.

The Examiner further states that the FLP/FRT recombination system does not work reliably in all plants. The Examiner points out that Lloyd et al. teach that FLP/FRT recombination did not work in Arabidopsis and that Luo et al. teach that in order to get the FLP/FRT system to work in Arabidopsis, use of an FLP gene sequence variations are required.

Applicant would like to point out that Lloyd et al. find the fact that recombination failed in Arabidopsis "interesting" and as such provide no apparent reason for this failure.

In addition, numerous studies reporting successful use of the FLP/FRT system in Arabidopsis have been published. For example, Sonti et al. (Plant molecular Biology 28:1127-1132., 1995) demonstrated that an FLP/FRT recombination system similar to that described by the present invention worked successfully in Arabidopsis (pg. 1130 paragraph 2 "FLP recombinase, like the mechanistically similar Cre recombinase ....." also demonstrated in Table 2: FLP activity in transgenic plants. Similar results were also demonstrated by Kilby et. al. [The plant Journal 1995 8 (5) 637-652]

Although Luo et al. report an improved FLP/FRT construct for use in Arabidopsis, the recombination results obtained thereby do not imply that the widely used FLP/FRT system which has been shown to be functional in numerous plant species including Arabidopsis would not function in any plant species. In fact, Luo et al. mention that functional recombination has been obtained (Kilby et al., Ibid.) and that variation in activity of this system in Arabidopsis may result from positional effects (Matzke and Matzke, 1998).

The Examiner further states that the instant application fails to provide guidance for selection of a first gene that can induce transcription through any transcription termination signals of the first gene to transcribe a second gene.

From these remarks it appears that the Examiner has not fully comprehended the utility of the present methodology. As is clearly described in the specification, expression of the second gene is enabled by the expression product of the first gene and following excision of the second gene and associated promoter from one chromosome of the chromosome pair.

Such excision removes the sequences flanked by the SSRS, and thus links the first promoter with the second gene through a short SSRS sequence. Following such excision and crossing with an unexcised plant, progeny plants include a first chromosome of a chromosome pair which includes the first promoter (operatively) linked to the second gene (from the excised parent), while the second chromosome of the pair includes all of the elements stated by the Examiner (from the unexcised parent). In the progeny plants, the expression product of the first gene (e.g., T7 polymerase) will activate (in trans) expression of the second gene from the first promoter (e.g., T7 promoter).

The Examiner states, with respect to claims 50-51 and 53, that given the lack of a written description in the specification with regards to structural and physical characteristics of the claimed plants, one skilled in the art would not have been in possession of the genus claimed at the time the application was made.

Applicant contends that this statement is in error. Claims 50-51 and 53 of the present invention describe plants characterized by exogenic allelism. Such plants are readily distinguishable from other non-transformed plants.

The distinguishing quality of the plants of the present invention is not the exogene(s) expressed thereby, but rather the allelic relationship formed between the two distinct exogenes expressed thereby.

Since such exogenic relationship stipulates that, for example, an expression product of one exogene activates a promoter of the second exogene

(claim 50), one of ordinary skill in the art could easily type the plants of the present invention by simply detecting the presence of both exogenes using standard molecular techniques such as PCR or Northern blotting.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 47, 49-51 and 55-57 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant has amended claims 47, 49-51 and 55 to replace the term "including" with --comprising--.

Applicant has also adopted Examiner's suggestions with respect to claims 47 (indefiniteness) and 49 ("a first and second plant") and as such appropriate amendments have been made to these claims.

Applicant has decided not to adopt the Examiners suggestions with regard to the description of the construct components and plants of claim 47 and other claims. Applicant believes that the claims, as presently written, are clear and concise and that no confusion exists as to what each component of the construct represents or the relationship between such components. Furthermore, it Applicant's strong belief that only the current state of the claims will accurately reflect that which is recited in the specification and illustrated in the drawings and as such claims amendments as suggested by the Examiner will, if any, generate confusion.

35 U.S.C. § 102(b) -Vergunst et al.

The Examiner has rejected claim 55 under 35 U.S.C. § 102(b) as anticipated by Vergunst et al.

The Examiner states that Vergunst et al teach plants that have different exogenes in an allelic relationship on two chromosomes of a chromosome pair, and that these genes would inherently segregate to different gametes. The Examiner further states that the construct described by Vergunst et al. is

analogous to the construct of Figure 1.

The Examiners rejections are respectfully traversed. Claim 55 has now been amended.

Claim 55 is directed at plant seeds which exhibit exogenic allelism. Exogenic allelism is defined in the instant application as "the allelic positioning of two functionally distinct exogenes on the chromosomes of a chromosome pair such that substantially 100 % segregation of the two exogenes is observed upon gamete formation".

Vergunst et al. use the Cre/lox system to target T-DNA into specific sites in the chromosomes. By using such a system Vergunst et al. claim to have generated plants which are homozygous for one gene while being hemizygous for another at a specific site of a chromosome.

Applicant contends that the plants generated by Vergunst et al. cannot exhibit true exogenic allelism nor can they exhibit complete segregation of exogenes into different gametes.

In the plants generated by Vergunst et al. one of the two exogenes used is expressed from both chromosomes (the bar gene) and as such functional segregation of this exogene from the other exogene in progeny plants will not occur in the gametes. Thus, although the constructs of Vergunst et al schematically resemble the construct illustrated in Figure 1, such schematic resemblance does not translate into functional similarities.

In addition, the system used by Vergunst et al. incorporates a recombinase expressing sequence within the transforming construct and two recombinase sites; presence of active recombinase and recombinase sites within the plant can lead to undesired and non-specific recombination events, thus leading to a less than optimal segregation of the two exogenes within gametes (see for example, the second paragraph of page 2732).

Not withstanding from the above, Applicant has elected to further limit the claims to include the term "functionally-hemizygotic" when referring to each of the two exogenes. As such, a functional (i.e., expressible) copy of each

exogene now exists only in one chromosome of the pair, a feature which is not described, suggested or enabled by the teachings of Vergunst et al.

35 U.S.C. § 102(b) - Fabijanski et al.

The Examiner states that claim 55 is rejected under 35 U.S.C. § 102(b) as being anticipated by Fabijanski et al.

The Examiner states that Fabijanski et al teach plants that have two different exogenes in allelic relationship are male sterile and fertile when crossed to a male fertile plant.

Applicant submits that this statement is in error. Fabijanski et al. teach plants which express a first gene capable of rendering a non-toxic compound toxic to cells critical for pollen formation and a second gene which encodes the non-toxic compound. Co-expression of such genes in the same plant renders the plant male-sterile while crossing such a male sterile plant with a male fertile plant would lead to recovered male fertility in some of the progeny plants as a result of a segregation of these two genes.

Fabijanski et al. do not teach nor do they suggest methods of generating true exogenic allelism of these two genes, and as such, the male-sterile plants described by Fabijanski et al. would not exhibit obligatory segregation of the two genes into different gametes. Therefore, when such male-sterile plants are crossed with the male fertile plants a fraction of the progeny plants will always be male-sterile.

Fabijanski et al. state that having these two genes on the same chromosome pair in each plant, preferably at the same genetic locus or position, would substantially reduce the chance of a crossing over event (column 15 lines 39-42), however, methods of generating such plants are neither described nor are they suggested.

Thus, in sharp contrast to the plants of the present invention, the male-sterile plants described by Fabijanski et al. will not exhibit obligatory segregation of the exogenes into different gametes, and thus would not

exhibit100% male fertility restoration when outcrossed.

35 U.S.C. § 103(a) - Llyod et al. in view of Snaith et al.

The Examiner has rejected claim 51 under 35 U.S.C. § 103(a) as being unpatentable over Llyod et al. in view of Snaith et al.

The Examiner states that Lloyd et al. teach plants with SSR containing constructs and suggest expressing two different site specific recombination systems in the same plant. Lloyd et al do not teach plasmids with multiples of different SSRSs.

Snaith et al. teach plasmids with multiple FRT and LoxP sites, suggest their use in combined manipulation strategies and discusses their importance as tools in in-vivo manipulation of DNA.

The Examiner further states that at the time the invention was made it would have been obvious to one ordinarily skilled in the art to produce plants with SSRS-containing constructs as taught by Lloyd et al., and to modify that to use constructs with two genes and two different sets of SSRSs as described by Snaith et al. The Examiner further states that one of ordinary skill in the art would have been motivated to do so because of the suggestion made by Lloyd et al. to use more than one site specific recombination system in the same plant.

Applicant contends that mere suggestion of use does not constitute motivation, especially where no utility is proposed for a scheme utilizing two distinct genes each flanked by a distinct recombination system.

It will be appreciated that the present invention does not claim that the use of a combination of recombinase systems in the same plant is novel, admitably, such use of recombinase systems has been previously reported. However, plants homozygous for an expression cassette which includes two distinct genes each flanked by a distinct recombination system have not been described nor have they been suggested by the prior art cited herein. The homozygous plants of the present invention are utilized as intermediates in a clever scheme of recombination excision and plant crossing and selection, which

generates plants exhibiting exogenic allelism.

. In sharp contrast, Lloyd et al. and Snaith et al. do not describe nor do they suggest plants homozygous for an expression cassette which includes two distinct genes each flanked by a distinct recombination system since no utility for such plants has been proposed or described in the prior art. As such, the teachings of Lloyd et al. and Snaith et al. would not motivate an ordinarily skilled artisan to generate the homozygous plants described in claim 51 of the instant application.

Not withstanding from the above and in interests of expediting prosecution of the instant application, Applicant has chosen to amend Claim 51 to include the limitation "said second transcribable polynucleotide sequence being selected such that an expression product thereof regulates an expression level of a product of said first transcribable polynucleotide sequence" further differentiating the present invention from the prior art cited.

Such a functional relationship between two gene sequences flanked by different recombination sequences is neither described nor is it suggested by Llyod et al. and/or Snaith et al.

In view of the above amendments and remarks it is respectfully submitted that claims 47, 49-51 and 55-57, are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

Sol Sheinbein
Attorney for Applicant
Registration No. 25,457

Date: May 30, 2001.

Enclosed:
One month extension fee
Version with marking to show changes made
Articles by:
Sonti et al.
Kilby et. al

VERSION WITH MARKING TO SHOW CHANGES MADE

In the claims:

Claims 47, 49-51 and 55 have now been amended as follows:

47. (Amended) A method of generating exogenic allelism in a plant, the method comprising the steps of:

(a) providing a first and a second ~~isogenic~~ plants isogenic for an expression cassette ~~including~~comprising:

(i) a first segment ~~including~~comprising a first promoter sequence;

(ii) a second segment ~~including~~comprising a first transcribable polynucleotide sequence; and

(iii) a third segment ~~including~~comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said third segment being flanked by said first and second segments, wherein a pair of site-specific recombination sequences are disposed one between said first segment and said third segment and another between said second segment and said third segment, such that said first promoter sequence is operatively coupled with said first transcribable polynucleotide sequence only following excision of said third segment from the expression cassette by site specific recombination via said pair of site-specific recombination sequences;

(b) introducing a recombinase into said first plant, so as to excise said third segment thereby operatively adjoining said first transcribable polynucleotide sequence to said first promoter sequence;~~and~~

(c) selfing a plant resultant from step (b) and selecting progeny which is recombinase minus;

(~~d~~e) crossing a plant resultant from step (b) and said second plant thereby obtaining an~~, so as to generate an~~ offspring- characterized by exogenic allelism.

49. (Amended) A method of generating exogenic allelism in a plant, the method comprising the steps of:

(a) providing~~—a—~~ first and second isogenic plants hemizygous or homozygous for an expression cassette ~~including~~comprising:

(i) a first segment ~~including~~comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, said first segment being flanked by a pair of first site-specific recombination sequences; and

(ii) a second segment, being linked to said first segment, said second segment ~~including~~comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said second segment being flanked by a pair of second site-specific recombination sequences;

(b) introducing a first recombinase into said first plant, so as to excise said first segment, and selfing said first plant and selecting progeny which is recombinase minus;

(c) introducing a second recombinase into said second plant, so as to excise said second segment, and selfing said second plant and selecting progeny which is recombinase minus; and-

(d) crossing a plant resultant from step (b) with a plant resultant from step (c), so as to generate an offspring characterized by exogenic allelism.

50. (Amended) A plant homozygous for an expression cassette ~~including~~comprising:

(a) a first segment ~~including~~comprising a first promoter sequence;

(b) a second segment ~~including~~comprising a first transcribable polynucleotide sequence; and

(c) a third segment ~~including~~comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said third segment being flanked by said first and second segments, wherein a pair of site-specific recombination sequences are disposed one between said first segment and said third segment and another between said second segment and said third segment, such that said first promoter sequence is operatively coupled with said first transcribable polynucleotide sequence only following excision of said third segment from the expression cassette by site specific recombination via said pair of site-specific recombination sequences;~~,~~

said second transcribable sequence being selected such that an expression product thereof activates said first promoter sequence to direct transcription of said first transcribable sequence.

51. (Amended) A plant homozygous for an expression cassette ~~including~~comprising:

(a) a first segment ~~including~~comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, said first segment being flanked by a pair of first site-specific recombination sequences; and

(b) a second segment, being linked to said first segment, said second segment ~~including~~comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said second segment being flanked by a pair of second site-specific recombination sequences, said second transcribable polynucleotide sequence being selected such that an expression product thereof regulates an expression level of a product of said first transcribable polynucleotide sequence.~~,~~

55. (Amended) Plant seeds each of which comprising a genome, said genome ~~including~~comprising a pair of exogenes, wherein a first exogene of said pair of exogenes ~~being~~ is located on a first chromosome of a chromosome pair of said genome of the plant seeds, and further wherein a second exogene of said pair of exogenes ~~being~~ is located on a second chromosome of said chromosome pair of said genome of the plant seeds, said first and said second exogenes being functionally-hemizygotic and in allelic relationship, such that said first and said second exogenes obligatorily segregate to different gametes.